Journal of Molecular Structure 1087 (2015) 1–10

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

Synthesis, structural characterization and anti-carcinogenic activity of new cyclotriphosphazenes containing dioxybiphenyl and chalcone groups

Ahmet Orhan Görgülü [a], Kenan Koran [a,*], Furkan Özen [a], Suat Tekin [b], Süleyman Sandal [b]

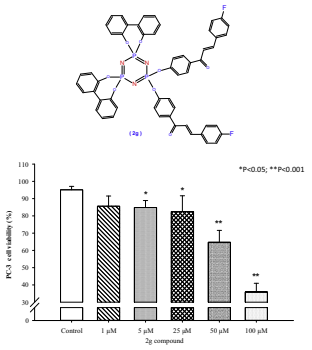
[a] Firat University, Faculty of Science, Department of Chemistry, 23169 Elazig, Turkey
[b] Inonu University, Faculty of Medicine, Department of Physiology, 44000 Malatya, Turkey

HIGHLIGHTS

- Compounds synthesized for the first time.
- And show antitumor activity.
- The effective dose is 100 μM.

GRAPHICAL ABSTRACT

The chalcone-cyclophosphazene compounds containing dioxybiphenyl groups (**2a–2h**) were synthesized. *In vitro* anti-carcinogenic activities of these compounds were performed by using MTT assay against PC-3 and LNCaP cancer cell lines. Results, these compounds (**2a–2h**) were found to have anti-tumor activity against PC-3 and LNCaP cancer cell lines.



ARTICLE INFO

Article history:
Received 26 November 2014
Received in revised form 15 January 2015
Accepted 17 January 2015
Available online 30 January 2015

Keywords:
Cyclotriphosphazene
Chalcone-phosphazenes
Anti-carcinogenic activity
PC-3 and LNCaP

ABSTRACT

2,2-Dichloro-4,4,6,6-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl]cyclotriphosphazene **(2)** was synthesized from hexachlorocyclotriphosphazene **(HCCP)** and 2,2′-dihydroxybiphenyl. The mixed substituent chalcone/dioxybiphenyl cyclophosphazenes (**2a–h**) were obtained from the reactions of **(2)** with hydroxy chalcone compounds in K_2CO_3/acetone system. The chalcone-cyclophosphazene compounds were characterized by elemental analysis, FT-IR, ^1H, ^{13}C, ^{31}P NMR techniques. *In vitro* anti-carcinogenic activities of all compounds were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Anti-carcinogenic activity of the compounds **(2a–h)** against androgen-dependent (LNCaP) and independent (PC-3) human prostate cancer cell lines were investigated. Our results indicate that the chalcone-phosphazene compounds **(2a–h)** have anti-carcinogenic activity on PC-3 and LNCaP cell lines ($p < 0.05$). The effective dose of the compounds was determined as 100 μM.

Introduction

Phosphazenes are molecules which contain —P=N— bonds. There are three important types of phosphazenes, such as linear,

* Corresponding author at: Department of Chemistry, Firat University, 23119 Elazig, Turkey. Fax: +90 424 2330062.
E-mail address: kumfosfit@gmail.com (K. Koran).

http://dx.doi.org/10.1016/j.molstruc.2015.01.033
0022-2860/

cyclic and poly. Trimer, tetramer and linear polyphosphazenes are the most known and studied types of phosphazenes [1].

The phosphazene derivatives have various physical and biological properties, for example liquid crystals [2,3], electrical conductivity [4], flame retardants [5–7], electrolytes for rechargeable batteries [8], fire resistant materials [9], dielectric properties [10], biomedical applications [11,12], antimicrobial, antibacterial [13–18], anti-leukemic [19] and strong anti-tumor activity [20–27].

Chalcones are compounds that can be prepared by the Claisen–Schmidt condensation reaction [28,29]. Because of the ketovinylenic group in chalcones and their analogs, they exhibit numerous physical and biological properties, for instance optical and fluorescence properties [30,31], dielectric properties [32,33], antioxidant and soybean lipoxygenase inhibitory activity [34], antimicrobial activity [35], Anti-HIV activity [36], antibacterial activity [37], anti-inflammatory [38] and anti-cancer activities [39–44].

The synthesis of different phosphazene compounds has been reported [13,45–53] but there are only four articles about synthesis of the phosphazene compounds bearing chalcone groups [10,54–56], there are, however, no studies about synthesis of dioxybiphenyl substituted chalcone-cyclophosphazene compounds. The cyclotriphosphazenes bearing 2,2′-dihydroxybiphenyl are much more stable to hydrolysis and thermal decomposition than hexachlorocyclotriphosphazene [1].

In this study, the chalcone compounds containing —OH groups were synthesized. And then these chalcone compounds **(1a–h)** were reacted with 2,2-dichloro-4,4,6,6,-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl)]cyclotriphosphazene in order to get substituted products. As a result, cyclophosphazenes bearing 2,2′-dioxybiphenyl groups and chalcone compounds were synthesized and characterized by elemental analysis, FT-IR, ^{1}H, ^{13}C, ^{31}P NMR techniques. Antitumor properties of these compounds were investigated by MTT ([3-(4,5-dimethylthiazol)-2-yl]-2,5-diphenyl-2H-tetrazolium bromide]) assay. The MTT assay is a simple procedure to determine living and growing cells without using radioactivity. Our results indicate that the chalcone-phosphazene compounds displayed potential antitumor activity towards on human prostate cancer cell lines (PC-3 and LNCaP).

Experimental

Materials and methods

Solvents and other liquids were purified by traditional methods. Hexachlorocyclotriphosphazene, $N_3P_3Cl_6$ (TCI), was crystallized from *n*-hexane. The chemicals were purchased from Merck and Sigma Aldrich. All reactions were monitored using thin-layer chromatography (TLC). The prostate carcinoma (PC-3 and LNCaP) and human breast (MCF-7) cancer cell lines were retrieved from the American Type Culture Collection (ATCC). Calf serum, trypsin, penicillin and streptomycin were purchased from Hyclone (Waltham, MA, USA).

FT-IR spectra were recorded on Perkin Elmer FT-IR spectrometer. Microanalysis was carried out by a LECO 932 CHNS-O apparatus. 1D (^{1}H, ^{13}C, ^{13}C APT and ^{31}P NMR) spectra were recorded using a Bruker DPX-400 spectrometer. The ^{1}H, ^{13}C and ^{31}P NMR chemical shifts were measured using TMS as an internal standard, whereas those for ^{31}P were measured using 85% H_3PO_4 as an external standard. For the NMR studies acetone-d6 was used as solvent for the compounds **2a** and **2d**. The chloroform-d was used as solvent for the compounds **2b**, **2c**, **2e**, **2f**, **2g** and **2h**.

Synthesis

4′-Hydroxy chalcone compounds were prepared by reaction of 4′-hydroxyacetophenone with various benzaldehydes [28,29]. 2,2-Dichloro-4,4,6,6-bis[spiro(2′,2″-dioxy-1″,1″-biphenyl]cyclotriphosphazene **(2)** was made as defined by Carriedo et al. [57]. The reaction of $[N_3P_3Cl_6]$ with the 2,2′-dihydroxybiphenyl took place under inert atmosphere.

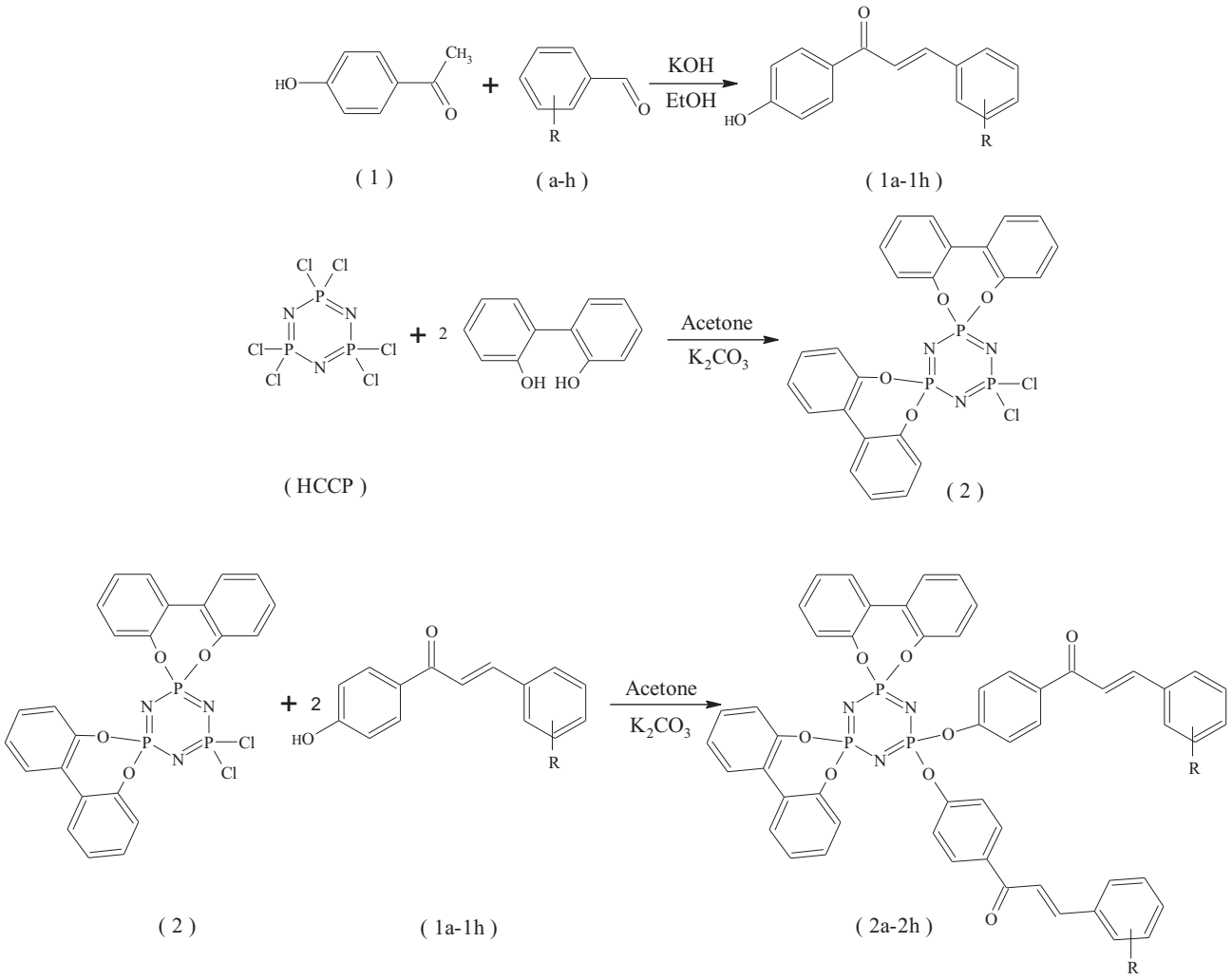
Preparation of substituted chalcone-phosphazenes

Chalcone-phosphazene compounds **(2a–2h)** were synthesized by similar methods; therefore, the experimental method for the synthesis of these compounds is only explained in detail for the first case.

*Synthesis of 2,2-(4′-oxychalcone)-4,4,6,6-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl] cyclotriphosphazene **(2a)**.* A mixture of compound **2** (1.0 g, 1.75 mmol) and K_2CO_3 (0.97 g, 7.0 mmol) in 50 mL dry acetone was slowly added, over 0.5 h, to a stirred solution of 4′-hydroxychalcone **(1a)** (0.9 g, 4.03 mmol) in 20 mL of dry acetone at 0 °C and then refluxed for 7 h. The solvent was evaporated. The residue was extracted with CH_2Cl_2 (4 × 25 mL) and then washed with 5% KOH solution four times and then dried over anhydrous magnesium sulfate. The solvent was concentrated on a rotary evaporator. After the solvent was removed, a white solid (**2a**) formed 1.49 g (90%). *Anal.* Calc. for $C_{54}H_{38}N_3O_8P_3$ (MW = 949.82): C, 68.28; H, 4.03; N, 4.42. Found: C, 68.02; H, 4.12; N, 4.49%. IR (KBr, cm^{-1}): 3061 and 3027 $\nu_{C-H(Ar.)}$, 2933 $\nu_{C-H(Aliphatic)}$, 1664 $\nu_{C=O}$, 1605, 1576 and 1567 $\nu_{C=C}$, 1175 and 1206 $\nu_{P=N}$, 1273 ν_{P-N-P}, 936 ν_{P-O-C}. ^{31}P NMR (Aceton-d_6) δ/ppm: 25.02 (2P, d, $P_a(O_2C_{12}H_8)$), 9.62 (1P, t, $P_b(O_4C_{30}H_{22})$). ^{1}H NMR (Aceton-d_6) δ/ppm: 8.40 (4H, d, H^9), 8.12 (4H, d, H^{13}), 7.98–7.76 (10H, m, H^{15}, H^{16} and H^{17}), 7.68 (2H, d, H^{12}), 7.62 (4H, d, H^3), 7.53–7.42 (8H, m, H^4 and H^5), 7.24 (4H, d, H^6), 7.0 (4H, d, H^8). ^{13}C NMR (Aceton-d_6) δ/ppm: 187.66 C^{11}, 153.94 C^7, 147.72 C^1, 144.06 C^{13}, 135.47 C^{14}, 134.90 C^{10}, 130.52 C^9, 129.88 C^5, 129.60 C^3, 128.76 C^{16}, 128.53 C^{15}, 128.32 C^2, 128.29 C^{17}, 126.33 C^4, 121.61 C^6, 121.13 C^{12}, 115.13 C^8.

*Synthesis of 2,2-(2′-oxy-2-methylchalcone)-4,4,6,6-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl]cyclotriphosphazene **(2b)**.* 4′-Hydroxy-2-methylchalcone **(1b)** (0.95 g, 4.03 mmol), 9 h. Yield: 1.27 g, 75%. *Anal.* Calc. for $C_{56}H_{42}N_3O_8P_3$ (MW = 977.87): C, 68.78; H, 4.33; N, 4.30. Found: C, 68.82; H, 4.26; N, 4.35%. IR (KBr, cm^{-1}): 3063 and 3027 $\nu_{C-H(Ar.)}$, 2947 and 2924 $\nu_{C-H(Aliphatic)}$, 1662 $\nu_{C=O}$, 1597, 1500 and 1477 $\nu_{C=C}$, 1175 and 1203 $\nu_{P=N}$, 1274 ν_{P-N-P}, 936 ν_{P-O-C}. ^{31}P NMR (chloroform-d) δ/ppm: 25.41 (2P, d, $P_a(O_2C_{12}H_8)$), 8.93 (1P, t, $P_b(O_4C_{32}H_{26})$). ^{1}H NMR (chloroform-d) δ/ppm: 8.15–8.20 (6H, m, H^9, H^{13}), 7.74 (2H, d, H^{12}), 7.54–7.56 (8H, m, H^3 and H^5), 7.52 (2H, d, H^{19}), 7.40–7.44 (4H, m, H^{17} and H^{18}), 7.33–7.37 (6H, m, H^4 and H^{16}), 7.28 (4H, d, H^6), 7.14 (4H, d, H^8), 2.51 (6H, s, H^{20}). ^{13}C NMR (chloroform-d) δ/ppm: 189.12 C^{11}, 154.29 C^7, 147.96 C^1, 142.76 C^{13}, 138.49 C^{15}, 135.35 C^{14}, 133.82 C^{10}, 130.99 C^{16}, 130.48 C^9, 130.43 C^{17}, 129.84 C^5, 129.71 C^3, 128.68 C^2, 126.46 C^4, 126.26 C^{19}, 122.72 C^{18}, 121.77 C^6, 121.30 C^{12}, 115.45 C^8, 19.92 C^{20}.

*Synthesis of 2,2-(4′-oxy-3-methylchalcone)-4,4,6,6-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl]cyclotriphosphazene **(2c)**.* 4′-Hydroxy-3-methylchalcone **(1c)** (0.95 g, 4.03 mmol), 8 h. Yield: 1.19 g, 70%. *Anal.* Calc. for $C_{56}H_{42}N_3O_8P_3$ (MW = 977.87): C, 68.78; H, 4.33; N, 4.30. Found: C, 68.70; H, 4.35; N, 4.39%. IR (KBr, cm^{-1}): 3062 and 3031$\nu_{C-H(Ar.)}$, 2954 and 2920$\nu_{C-H(Aliphatic)}$, 1663 $\nu_{C=O}$, 1601, 1584, 1500 and 1477$\nu_{C=C}$, 1175 and 1201$\nu_{P=N}$, 1273ν_{P-N-P}, 936 ν_{P-O-C}. ^{31}P NMR (chloroform-d) δ/ppm: 24.83 (2P, d, $P_a(O_2C_{12}H_8)$), 8.99 (1P, t, $P_b(O_4C_{32}H_{26})$). ^{1}H NMR (chloroform-d) δ/ppm: 8.14–8.16 (6H, m, H^9, H^{13}), 7.83 (2H, d, H^{12}), 7.54–7.58 (8H, m, H^3 and H^5), 7.49 (2H, d, H^{19}), 7.40–7.44 (4H, m, H^{17} and H^{18}), 7.32–7.37 (6H, m, H^4 and H^{15}), 7.28 (4H, d, H^6), 7.14 (4H, d, H^8), 2.43 (6H, s, H^{20}). ^{13}C NMR (chloroform-d) δ/ppm: 189.29 C^{11}, 154.25 C^7, 147.97 C^1, 145.40 C^{13}, 138.70 C^{16}, 135.38 C^{14}, 134.73 C^{10}, 131.57 C^{15}, 130.47



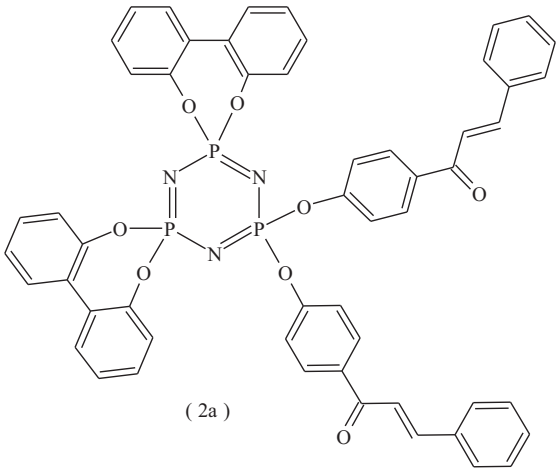
Scheme 1. General presentation of the all reactions.

C^9, 129.84 C^5, 129.71 C^3, 129.10 C^{17}, 128.90 C^{18}, 128.68 C^2, 126.25 C^4, 125.81 C^{19}, 121.77 C^6, 121.30 C^{12}, 115.43 C^8, 21.37 C^{20}.

Synthesis of 2,2-(4′-oxy-4-methylchalcone)-4,4,6,6-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl] cyclotriphosphazene ***(2d)****.* 4′-Hydroxy-4-methylchalcone **(1d)** (0.95 g, 4.03 mmol), 6 h. Yield: 1.46 g, 86%. *Anal.* Calc. for $C_{56}H_{42}N_3O_8P_3$ (MW = 977.87): C, 68.78; H, 4.33; N, 4.30. Found: C, 68.72; H, 4.35; N, 4.38%. IR (KBr, cm^{-1}): 3063 and 3027 $\nu_{C-H(Ar.)}$, 2950 $\nu_{C-H(Aliphatic)}$, 1662 $\nu_{C=O}$, 1602, 1567 and 1500 $\nu_{C=C}$, 1173 and 1200 $\nu_{P=N}$, 1273 ν_{P-N-P}, 936 ν_{P-O-C}. ^{31}P NMR (Aceton-d_6) δ/ppm: 25.03 (2P, d, $P_a(O_2C_{12}H_8)$), 9.63 (1P, t, $P_b(O_4C_{32}H_{26})$). ^1H NMR (Aceton-d_6) δ/ppm: 8.38 (4H, d, H^9), 8.11 (2H, d, H^{13}), 7.67 (2H, d, H^{12}), 7.61 (4H, d, H^3), 7.53–7.41 (8H, m, H^4 and H^5), 7.44 (4H, d, H^{15}), 7.23–7.21 (8H, m, H^6 and H^{16}), 7.02 (4H, d, H^8), 2.39 (6H, s, H^{18}). ^{13}C NMR (Aceton-d_6) δ/ppm: 187.64 C^{11}, 153.87 C^7, 147.72 C^1, 144.15 C^{13}, 140.81 C^{17}, 135.59 C^{14}, 132.18 C^{10}, 130.46 C^9, 129.88 C^5, 129.60 C^3, 129.44 C^{16}, 128.58 C^2, 128.33 C^{15}, 126.33 C^4, 121.65 C^6, 121.10 C^{12}, 115.11 C^8, 20.42 C^{18}.

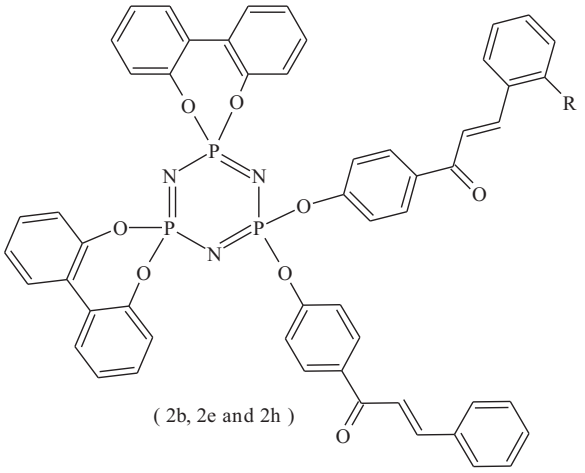
Synthesis of 2,2-(4′-oxy-2-fluorochalcone)-4,4,6,6-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl] cyclotriphosphazene ***(2e)****.* 4′-Hydroxy-2-fluorochalcone **(1e)** (1 g, 4.03 mmol), 5 h. Yield: 1.55 g, 90%. *Anal.* Calc. for $C_{54}H_{36}F_2N_3O_8P_3$ (MW = 985.80): C, 65.79; H, 3.68; N, 4.26. Found: C, 65.83; H, 3.73; N, 4.22%. IR (KBr, cm^{-1}): 3067 and 3041 $\nu_{C-H(Ar.)}$, 2962 and 2924 $\nu_{C-H(Aliphatic)}$, 1665 $\nu_{C=O}$, 1598, 1507 and 1476 $\nu_{C=C}$, 1175 and 1204 $\nu_{P=N}$, 1274 ν_{P-N-P}, 936 ν_{P-O-C}. ^{31}P NMR (chloroform-d) δ/ppm: 25.02 (2P, d, $P_a(O_2C_{12}H_8)$), 9.62 (1P, t, $P_b(O_4C_{30}H_{20})$). ^1H NMR (chloroform-d) δ/ppm: 8.13–8.17 (6H, m, H^9, H^{13}), 7.83 (2H, d, H^{12}), 7.31–7.66 (20H, m, H^3, H^4, H^5, H^{16}, H^{17}, H^{18} and H^{19}), 7.12–7.18 (8H, m, H^6 and H^8). ^{13}C NMR (chloroform-d) δ/ppm: 188.97 C^{11}, 165.40 and 162.89 C^{15}, 154.31 C^7, 147.90 C^1, 143.84 C^{13}, 135.24 C^{14}, 131.04 C^{10}, 130.50 C^9, 130.46 C^{17}, 129.85 C^5, 129.74 C^3, 128.67 C^2, 126.46 C^4, 121.75 C^6, 121.37–121.32 C^{18} and C^{19}, 121.27 C^{12}, 116.32 C^8, 116.10 C^{16}.

Synthesis of 2,2-(4′-oxy-3-fluorochalcone)-4,4,6,6-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl]cyclotriphosphazene ***(2f)****.* 4′-Hydroxy-3-fluorochalcone **(1f)** (1 g, 4.03 mmol), 5 h. Yield: 1.21 g, 70%. *Anal.* Calc. for $C_{54}H_{36}F_2N_3O_8P_3$ (MW = 985.80): C, 65.79; H, 3.68; N, 4.26. Found: C, 65.73; H, 3.72; N, 4.30%. IR (KBr, cm^{-1}): 3065 and 3038$\nu_{C-H(Ar.)}$, 2962 and 2925$\nu_{C-H(Aliphatic)}$, 1666 $\nu_{C=O}$, 1608, 1582, 1501 and 1477$\nu_{C=C}$, 1174 and 1201$\nu_{P=N}$, 1273ν_{P-N-P}, 936 ν_{P-O-C}.



(2a)

R : -H, for compound 2a

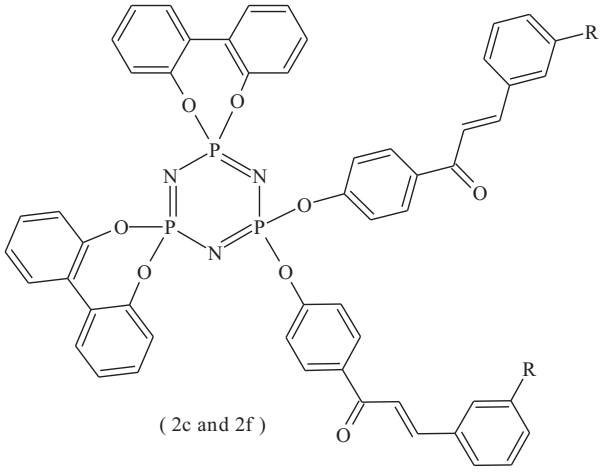


(2b, 2e and 2h)

R : 2-CH_3, for compound 2b

R : 2-F, for compound 2e

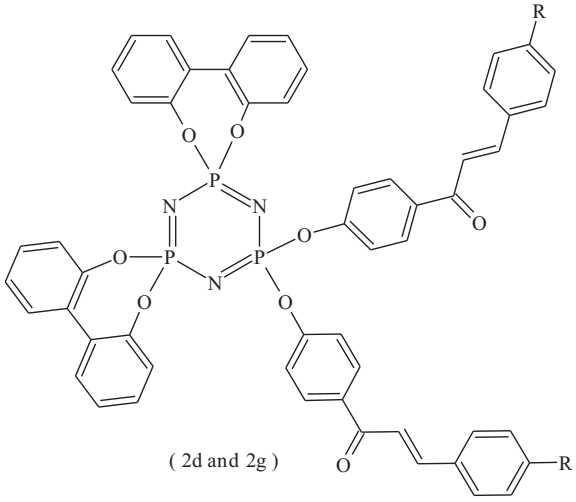
R : 2-Cl, for compound 2h



(2c and 2f)

R : 3-CH_3, for compound 2c

R : 3-F, for compound 2f



(2d and 2g)

R : 4-CH_3, for compound 2d

R : 4-F, for compound 2g

Scheme 2. Structures of the compounds **2a**–**2h**.

^{31}P NMR (chloroform-d) δ/ppm: 24.79 (2P, d, $P_a(O_2C_{12}H_8)$), 8.91 (1P, t, $P_b(O_4C_{30}H_{20})$). ^{1}H NMR (chloroform-d) δ/ppm: 8.42 (4H, d, H^9), 8.32 (2H, s, H^{15}), 8.10 (2H, d, H^{13}), 7.88 (2H, d, H^{19}), 7.80 (2H, d, H^{12}), 7.71 (2H, t, H^{18}), 7.44–7.58 (16H, m, H^3, $H^4$and H^5), 7.28–7.33 (6H, m, H^6 and H^{17}), 7.23 (4H, d, H^8). ^{13}C NMR (chloroform-d) δ/ppm: 188.83 C^{11}, 164.29 and 161.84 C^{16}, 154.34 C^7, 147.94 C^1, 143.62 C^{13}, 137.06 C^{15}, 135.05 C^{14}, 130.63–130.53 C^9 and C^{10}, 129.85 C^5, 129.75 C^3, 128.67 C^2, 126.30 C^4, 124.63 C^{19}, 122.80 C^{18}, 121.73 C^6, 121.32 C^{12}, 117.44–117.65 C^{17}, 114.45–114.66 C^8.

Synthesis of 2,2-(4′-oxy-4-fluorochalcone)-4,4,6,6-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl]cyclotriphosphazene ***(2g)***. 4′-Hydroxy-4-fluorochalcone **(1g)** (1 g, 4.03 mmol), 5 h. Yield: 1.52 g, 88%. *Anal.* Calc. for $C_{54}H_{36}F_2N_3O_8P_3$ (MW = 985.80): C, 65.79; H, 3.68; N, 4.26. Found: C, 65.81; H, 3.60; N, 4.33%. IR (KBr, cm^{-1}): 3065 and 3038 $\nu_{C-H(Ar.)}$, 2969 and 2927 $\nu_{C-H(Aliphatic)}$, 1666 $\nu_{C=O}$, 1605, 1576, 1500 and 1477 $\nu_{C=C}$, 1175 and 1.202 $\nu_{P=N}$, 1275 ν_{P-N-P}, 936 ν_{P-O-C}. ^{31}P NMR (chloroform-d) δ/ppm: 24.80 (2P, d, $P_a(O_2C_{12}H_8)$), 8.92 (1P, t, $P_b(O_4C_{30}H_{20})$). ^{1}H NMR (chloroform-d) δ/ppm: 8.14–17 (4H, d, H^9), 7.98 (2H, d, H^{13}), 7.71 (2H, d, H^{12}), 7.54–7.56 (8H, m, H^3 and H^{15}), 7.33–7.44 (8H, m, H^4 and H^5), 7.17–7.25 (8H, m, H^6 and H^{16}), 7.14 (4H, d, H^8).

^{13}C NMR (chloroform-d) δ/ppm: 189.14 C^{11}, 160.54 and 160.03 C^{17}, 154.38 C^7, 147.96 C^1, 137.86 C^{13}, 135.13 C^{14}, 132.03 C^{10}, 130.55 C^9, 129.95 C^{15}, 129.84 C^5, 129.71 C^3, 129.68 C^2, 126.26 C^4, 121.77 C^6, 121.34 C^{12},116.47 C^{16}, 116.25 C^8.

Synthesis of 2,2-(4′-oxy-2-chlorochalcone)-4,4,6,6-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl]cyclotriphosphazene ***(2h)***. 4′-Hydroxy-2-chlorochalcone **(1h)** (0.9 g, 3.48 mmol), 10 h. Yield: 1.15 g, 65%. *Anal.* Calc. for $C_{54}H_{36}Cl_2N_3O_8P_3$ (MW = 1018.71): C, 63.67; H, 3.56; N, 4.12. Found: C, 63.72; H, 3.50; N, 4.18%. IR (KBr, cm^{-1}): 3065 and 3031 $\nu_{C-H(Ar.)}$, 2960 and 2925 $\nu_{C-H(Aliphatic)}$, 1665 $\nu_{C=O}$, 1602, 1564 and 1500 $\nu_{C=C}$, 1179 and 1207 $\nu_{P=N}$, 1272 ν_{P-N-P}, 935 ν_{P-O-C}. ^{31}P NMR (chloroform-d) δ/ppm: 25.38 (2P, d, Pa($O_2C_{12}H_8$)), 9.48 (1P,

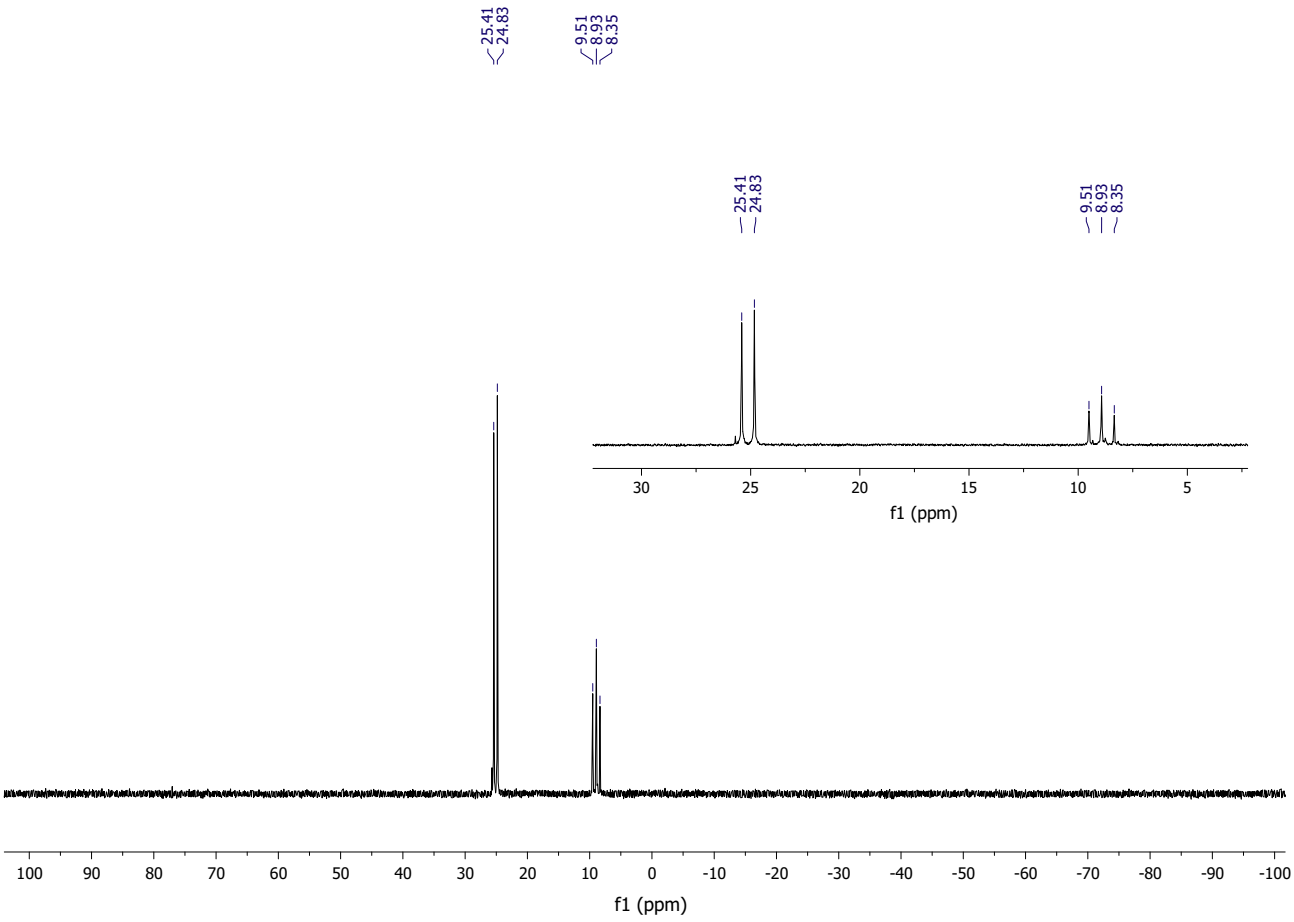


Fig. 1. ^{31}P NMR spectrum of compound **2b** (chloroform-d).

t, $Pb(O_4C_{30}H_{20})$). ^{1}H NMR (chloroform-d) δ/ppm: 8.26 (2H, d, H^{13}), 8.15 (4H, d, H^9), 7.79 (2H, dd, H^{19}), 7.32–7.56 (24H, m, H^3, H^4, H^5, H^6, H^{12}, H^{16}, H^{17} and H^{18}), 7.14 (4H, d, H^8). ^{13}C NMR (chloroform-d) δ/ppm: 189.08 C^{11}, 154.39 C^7, 147.95 C^1, 140.91 C^{13}, 135.57 C^{15}, 135.05 C^{14}, 133.17 C^{16}, 131.30 C^{10}, 130.60 C^9, 130.36 C^{17}, 129.84 C^5, 129.71 C^3, 128.68 C^2, 127.85 C^{19}, 127.13 C^{18}, 126.26 C^4, 121.76 C^6, 121.28 C^{12}, 117.22 C^8.

In vitro anticancer activity

Human breast cancer (MCF-7) and human prostate cancer (PC-3 and LNCaP) cell lines were preserved in Dulbecco's modified Eagle's medium (DMEM) culture medium supplemented with 4 mM L-glutamine, with 4500 mg/L glucose (10% heat-inactivated fetal bovine serum, 100 U/mL penicillin–streptomycin), with addition of 10 mM non-essential amino acids for culture of breast cancer cells. The cell lines were preserved at 37 °C in 5% CO_2 humidified incubator. The cytotoxicity effects of phosphazene compounds were determined against human breast cancer (MCF-7) and human prostate cancer (PC-3 and LNCaP) cell lines by using MTT ([3-(4,5-dimethylthiazol)-2-yl]-2,5-diphenyl-2H-tetrazolium bromide]) assay method [58–61].

The yellow MTT was transformed to a dark blue formazan product that was determined by a micro plate reader. The MTT assay is a simple procedure to determine living and growing cells. Breast and prostate cancer cells were plated in triplicate in 96-well flat bottom tissue culture plates. These cells treated with different concentrations (1, 5, 25, 50 and 100 μM) of the chalcone-phosphazene compounds. The culture plate cells were incubated for 24 h at 37 °C in 5% CO_2 humidified incubator. MTT (0.005 g/mL in phosphate buffer saline) was added to the cell culture and incubated for 4 h. The formazan that occurred from the reaction of mitochondria with MTT were dissolved in isopropanol (0.04 N 100 mL). All plates were read at 570 nm by micro plate reader (Biotek Synergy). Each data point is reported as an average of 10 measurements. All cellular results were measured against control cells [58–61].

All data were expressed as mean ± SD. Normality was tested by Shapiro Wilk Test. Homogeneity of variances was measured using Levene's method. Groups were compared by one-way analysis of variance. Because of nonhomogeneity of variances, Tamhane T2 test was used for multiple comparisons. $P < 0.05$ was considered as significant.

Results and discussion

Synthesis

4′-hydroxy chalcones **(1a–h)** were obtained from the reaction of 4′-hydroxyacetophenone with substitute benzaldehydes [28,29].

2,2-Dichloro-4,4,6,6-bis[spiro(2′,2″-dioxy-1″,1″-biphenyl]cyclotriphosphazene **(2)** was synthesized from the reaction of hexachlorocyclotriphosphazene **(HCCP)** with 2,2′-dihydroxybiphenyl under dry argon [54]. The reactions of **(2)** with 2.1 equiv. of hydroxy chalcones in the presence of K_2CO_3 in acetone gave the substituted products (**2a–h**). The chalcone-phosphazene compounds were generally obtained in high yields. These compounds were characterized by elemental analysis, FT-IR, ^{1}H, ^{13}C, ^{31}P NMR spectroscopy techniques. General presentation of the reactions is shown in Scheme 1 and structures of the compounds **2a–2h** are shown in Scheme 2.

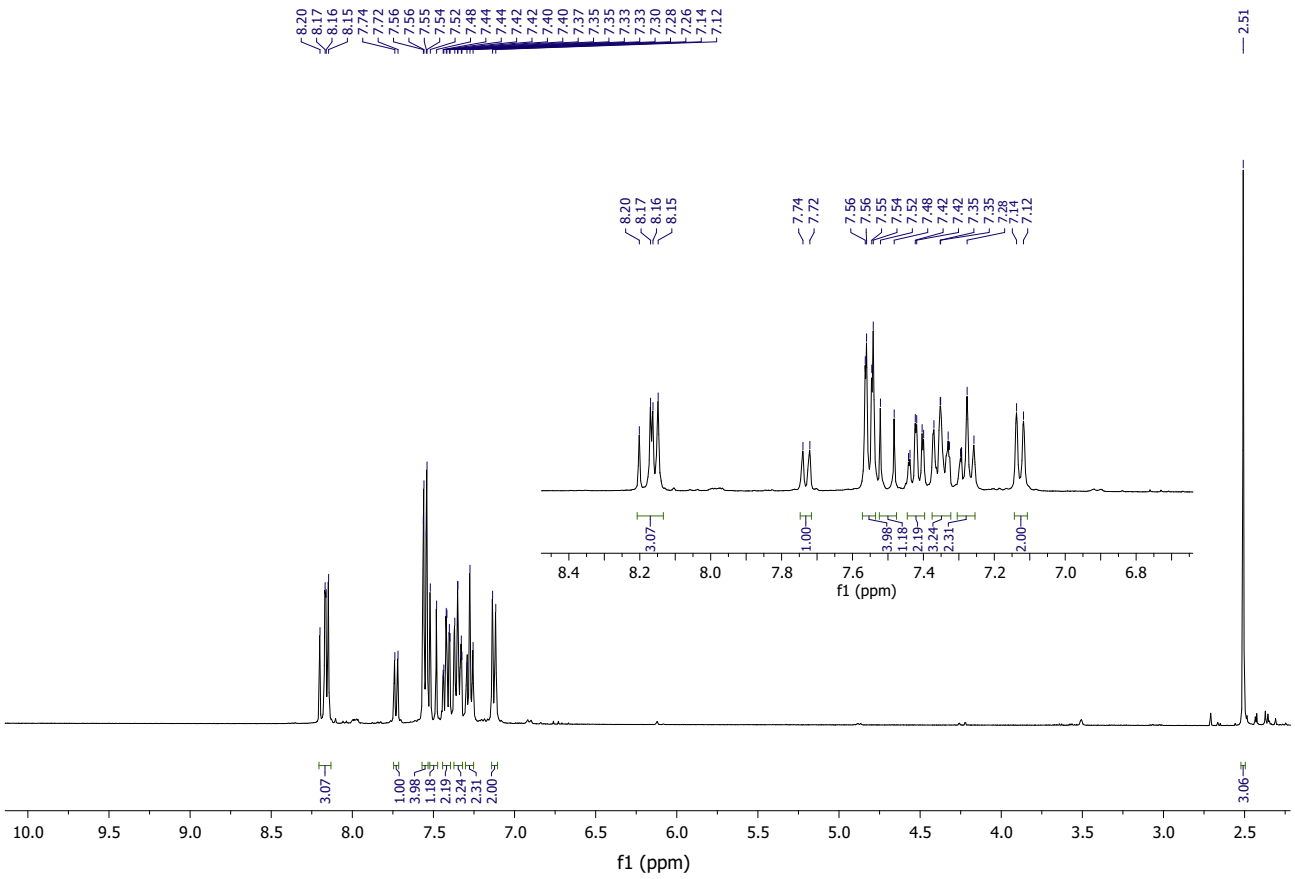


Fig. 2. ^{1}H NMR spectrum of compound **2b** (chloroform-d).

FT-IR spectroscopy

The —P═N stretching vibrations, which are observed between 1173 and 1207 cm^{-1}, are characteristic of the cyclophosphazene compounds. The absence of the OH stretching vibration in the FT-IR spectra of **2a**–**2h** indicates that all hydrogen atoms of the OH groups have been replaced. In the FT-IR spectra of **2a**–2**h**, the P—O—C stretching vibrations which were observed between 935 and 937 cm^{-1} and the C═O stretching vibrations which were observed between 1662 and 1666 cm^{-1} also indicate the substitute chalcone-phosphazene compounds.

NMR spectroscopy

The ^{31}P NMR data for **2a–2h** are given in experimental section (AB_2 system). There are two peaks in the ^{31}P NMR spectra of chalcone substituted phosphazene compounds **(2a–2h)**. The ^{31}P NMR spectra of **2a–2h** give two sets of peaks around δ = 8.91 and 25.41 ppm in a triplet-doublet. The ^{31}P NMR spectrum of **2b** is depicted in Fig. 1.

The ^{1}H and ^{13}C NMR data also confirm the structures of **2a**–**2h** (Scheme 2). In the ^{1}H NMR spectra of **2a–2h**, the absence of the OH protons indicates the chalcone substitute phosphazene products. The ^{1}H NMR spectra of **2b** is depicted in Fig. 2. The methyl protons for the compounds **2b**, **2c**, and **2d** were observed at 2.51, 2.43 and 2.39 ppm respectively. The aromatic protons for all the compounds appear between 7.0 and 8.42 ppm. —OH peaks of the chalcone groups were not observed in ^{1}H NMR spectrum of compounds **2a–2h**.

The detailed ^{13}C NMR spectral data were given in experimental section. The ^{13}C NMR spectrum of **2b** is depicted in Fig. 3 as an example. The carbonyl carbon atoms (C═O, C^{11}) for **2a–2h** were observed at 187.66, 189.12, 189.29, 187.64, 188.97, 188.83, 189.14 and 189.08 ppm, respectively. The methyl carbons for the compounds **2b**, **2c** and **2d** were observed at 19.92, 21.37 and 20.42 ppm respectively. For the compounds **2a–2h**, the aliphatic carbons which were numbered as 12 in all compounds were observed at 121.13, 121.30, 121.30, 121.10, 121.27, 121.12, 121.32 and 121.34 ppm respectively, while the aliphatic carbons which were numbered as 13 in all compounds were observed at 144.06, 142.76, 145.40, 144.15, 143.84, 143.62, 137.86 and 140.61 ppm respectively.

In vitro anti-tumor activity

The chalcone-phosphazene compounds synthesized were tested for their *in vitro* anti-tumor activity against three cancer cell lines: MCF-7 (human breast cancer cells), LNCaP (androgen-dependent human prostate cancer cells) and PC-3 (androgen-independent human prostate cancer cells) at five different concentrations (1, 5, 25, 50 and 100 μM) by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. The % cell viability of tested chalcone-phosphazene compounds are presented in Tables 1 and 2. Figs. 4 and 5 shows the effects of the chalcone-phosphazene compounds on cell viability measured at 24 h after exposure.

The chalcone-phosphazene compounds **(2a–h)** have anti-carcinogenic activity on PC-3 and LNCaP cell lines ($p < 0.05$). At 100 μM concentrations of all the compounds significantly reduced the percentage of viability of PC-3 and LNCaP cells ($^{**}p < 0.001$). The compounds **2d** (*p*-methyl) showed more potent activity than the compounds **2b** (*o*-methyl) and **2c** (*m*-methyl) against PC-3 cell lines (Table 1). The compounds **2b** (*o*-methyl) and **2c** (*m*-methyl)

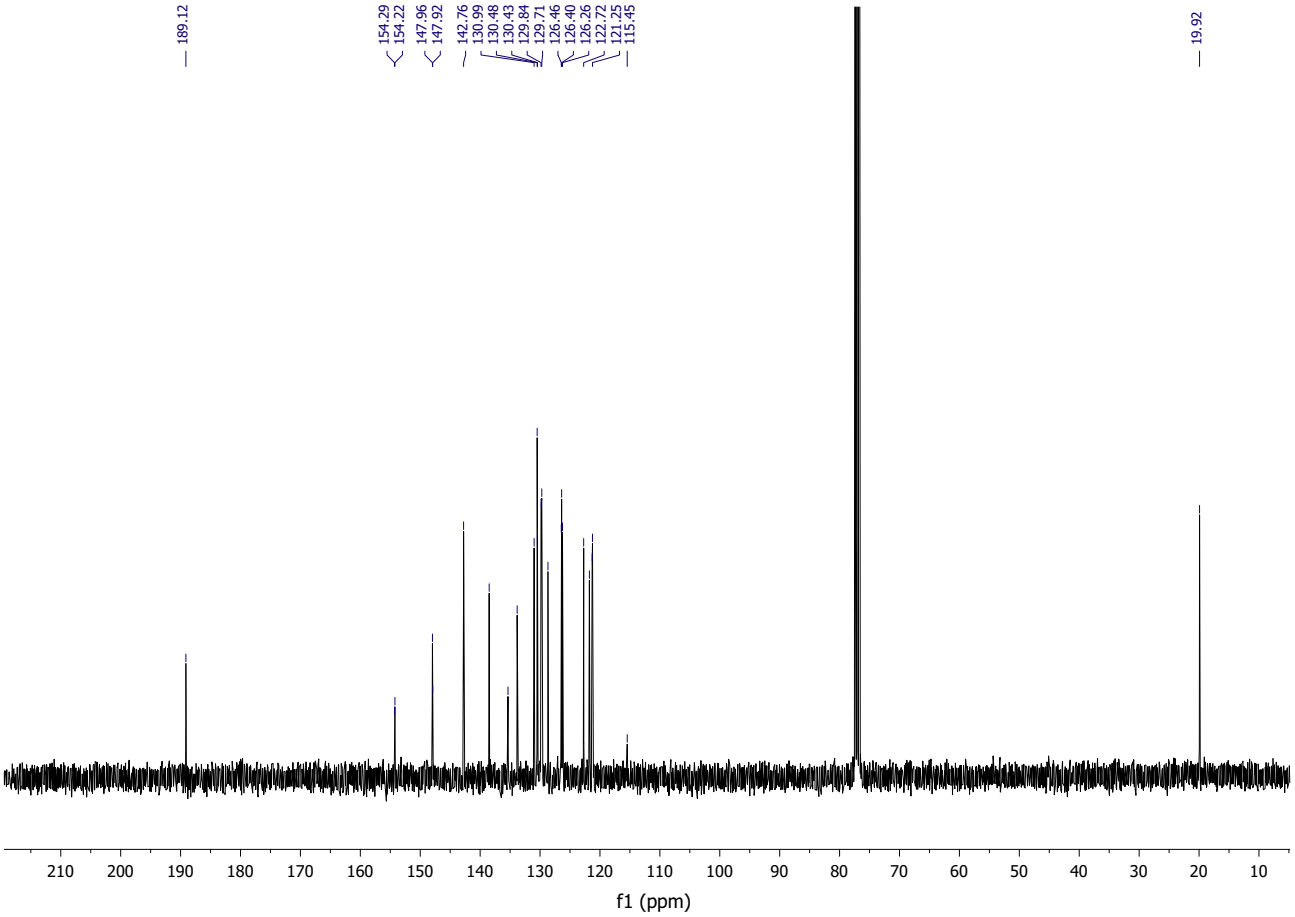


Fig. 3. ^{13}C NMR spectrum of compound **2b** (chloroform-d).

Table 1
Dose dependent cell-viability results in PC-3 cells after exposure to the chalcone-phosphazene compounds **(2a–2h)**.

Groups	Control	1 μM	5 μM	25 μM	50 μM	100 μM
2a	95.13 ± 1.99	88.27 ± 4.28	85.35 ± 5.01*	82.94 ± 2.97*	69.33 ± 4.01**	47.06 ± 3.11**
2b	95.13 ± 1.99	86.73 ± 7.06	86.25 ± 6.83	82.46 ± 5.4*	62.56 ± 2.42**	42.36 ± 6.49**
2c	95.13 ± 1.99	88.78 ± 6.41	89.61 ± 4.95	88.40 ± 7.23	80.57 ± 4.49**	54.56 ± 3.39**
2d	95.13 ± 1.99	87.33 ± 3.40*	87.49 ± 4.06*	87.43 ± 3.44*	73.84 ± 4.02**	52.28 ± 4.33**
2e	95.13 ± 1.99	88.28 ± 2.57	85.91 ± 5.39*	82.45 ± 4.08*	73.97 ± 2.92**	54.56 ± 3.39**
2f	95.13 ± 1.99	82.78 ± 5.0*	83.88 ± 3.34*	81.73 ± 6.86*	64.83 ± 6.1**	43.71 ± 3.02**
2g	95.13 ± 1.99	85.65 ± 5.9	84.87 ± 4.04*	82.53 ± 9.16*	64.71 ± 6.92**	35.93 ± 5.04**
2h	95.13 ± 1.99	90.12 ± 4.18	90.97 ± 6.18	88.07 ± 5.62*	66.57 ± 2.41**	51.45 ± 4.97**

* $p < 0.05$.
** $p < 0.001$.

Table 2
Dose dependent cell-viability results in LNCaP cells after exposure to the chalcone-phosphazene compounds **(2a–2h)**.

Groups	Control	1 μM	5 μM	25 μM	50 μM	100 μM
2a	93.37 ± 2.39	87.98 ± 4.84	84.07 ± 13.72*	80.04 ± 5.66*	79.84 ± 5.85**	62.51 ± 2.53**
2b	93.37 ± 2.39	77.93 ± 8.84*	75.05 ± 9.87*	74.95 ± 4.16*	69.55 ± 5.67**	69.04 ± 5.95**
2c	93.37 ± 2.39	88.58 ± 7.94	87.85 ± 2.50	83.79 ± 5.42*	82.21 ± 4.47*	77.83 ± 4.54**
2d	93.37 ± 2.39	86.06 ± 3.95	87.23 ± 6.11	91.13 ± 16.01	77.98 ± 9.86*	68.14 ± 6.37**
2e	93.37 ± 2.39	80.65 ± 5.96*	80.47 ± 9.42*	78.71 ± 6.01*	77.37 ± 6.41**	63.66 ± 2.67**
2f	93.37 ± 2.39	81.53 ± 4.82*	79.88 ± 7.65*	81.97 ± 4.45*	78.42 ± 9.67*	63.78 ± 3.43**
2g	93.37 ± 2.39	84.30 ± 10.47*	84.19 ± 3.79*	77.15 ± 12.85*	68.62 ± 5.66**	57.66 ± 6.36**
2h	93.37 ± 2.39	89.37 ± 7.50	87.69 ± 4.69	86.90 ± 5.19	76.16 ± 3.88**	64.79 ± 6.36**

* $p < 0.05$.
** $p < 0.001$.

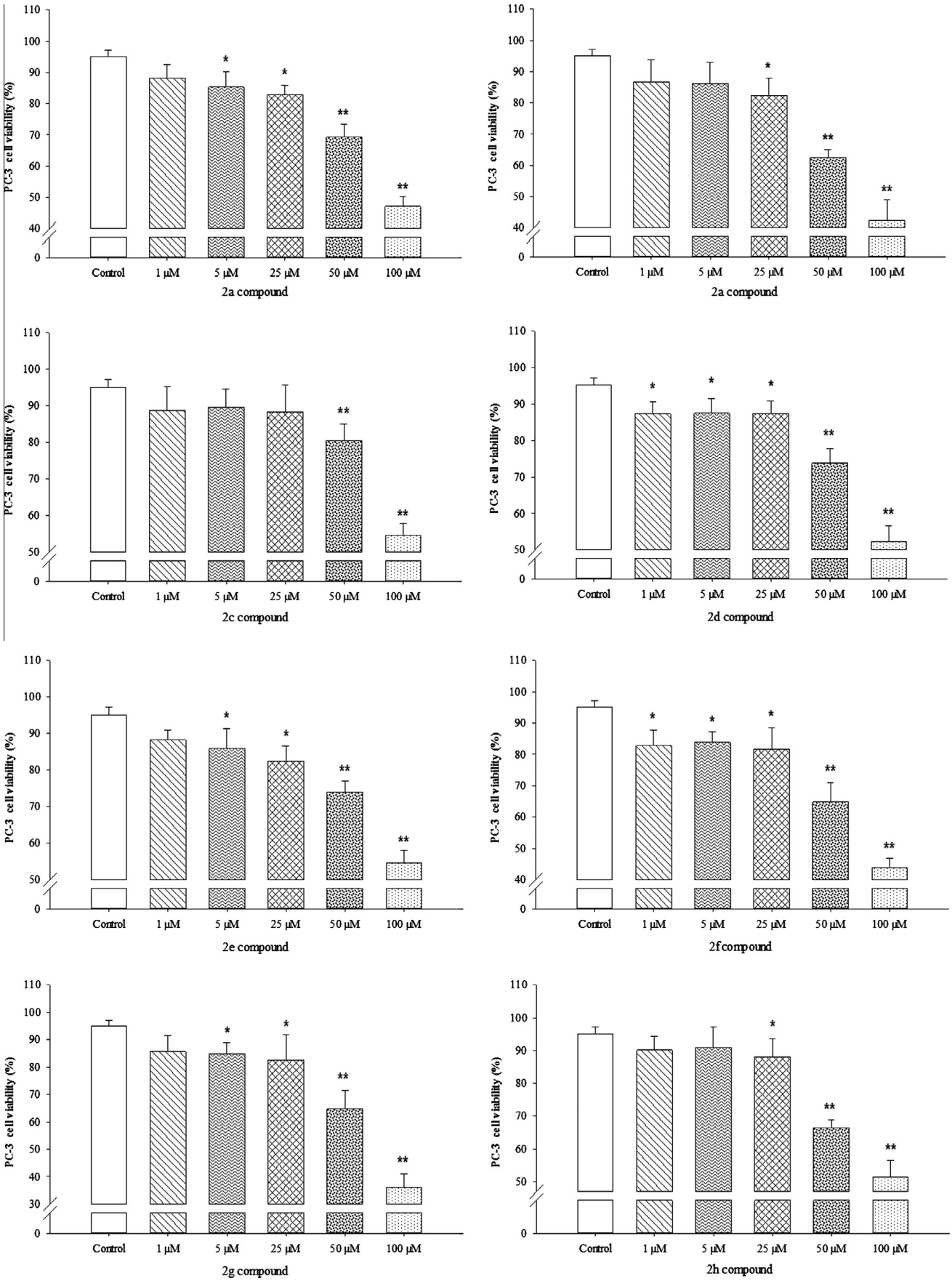


Fig. 4. The relative cell viability (%) of PC-3 cells following the exposure of various concentrations of all the compounds **(2a–2h)** and untreated control cell for 24 h ($^{*}p < 0.05$; $^{**}p < 0.001$).

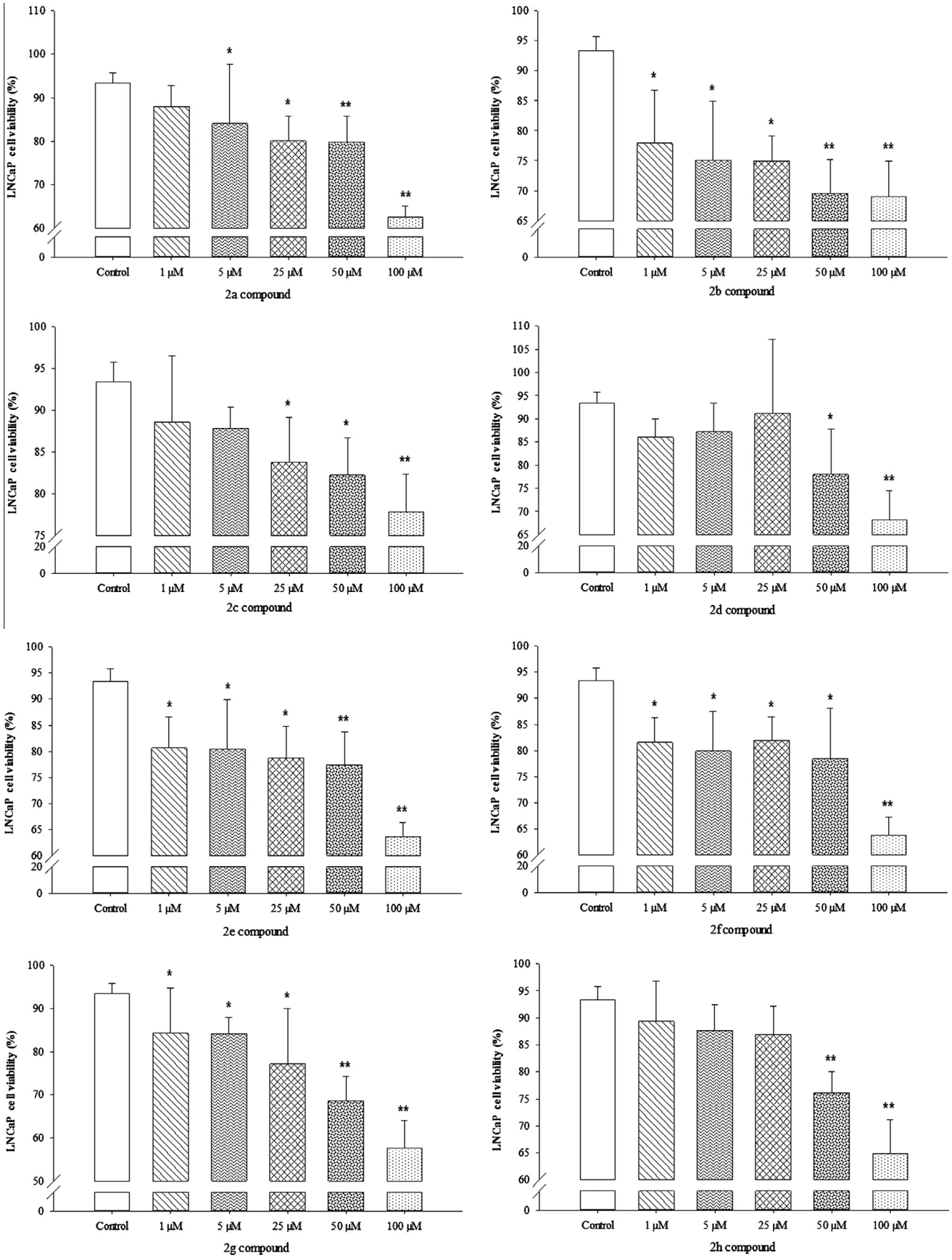


Fig. 5. The relative cell viability (%) of LNCaP cells following the exposure of various concentrations of all the compounds **(2a–2h)** and untreated control cell for 24 h ($^{*}p < 0.05$; $^{**}p < 0.001$).

showed more potent activity than the compounds **2d** (*p*-methyl) against LNCaP cell lines (Table 2). The chalcone-phosphazene compounds **(2e**, **2f and 2g)** bearing a fluorine atom exhibited better activity than other the chalcone-phosphazene compounds. In general, chalcone derivatives exhibit anti-cancer activity [39–44]. But, there are no studies about anti-cancer properties of chalcone-cyclophosphazene compounds. Our study of chalcone-cyclophosphazenes is the first on human breast (MCF-7) and prostate (LNCaP and PC-3) cancer cells. These results displayed that cyclophosphazene bearing chalcone compounds may be used as chemotherapy drug.

Conclusions

In summary, the chalcone-cyclophosphazene compounds **(2a–2h)** containing dioxybiphenyl groups were synthesized for the first time by using of K_2CO_3/acetone system. All chalcone-phosphazene compounds **(2a–2h)** were generally resulted in high yields. The synthesized chalcone phosphazene compounds were characterized by elemental analysis, FT-IR, ^{31}P, ^{1}H, ^{13}C NMR techniques. All chalcone-phosphazene compounds **(2a–2h)** were evaluated *in vitro* for their anticancer activity by MTT assay. The chalcone-phosphazene compounds **(2a–2h)** have not antitumor activity on MCF-7 ($p > 0.05$). All compounds showed highest antitumor activity against PC-3 and LNCaP cell lines ($p < 0.001$). These results displayed that cyclophosphazene bearing chalcone compounds may be useful for anticancer drug development in the future.

Acknowledgement

Firat University would like to thank for their support.

References

[1] H.R. Allcock, Phosphorus-Nitrogen Compounds: Cyclic, Linear and Polymeric Systems, Academic Press Inc., New York, 1972.
[2] J. Barbera, M. Bardaji, J. Jimenez, A. Laguna, M.P. Martınez, L. Oriol, J.L. Serrano, I. Zaragozano, J. Am. Chem. Soc. 127 (2005) 8994–9002.
[3] K. Moriya, T. Suzuki, S. Yano, S. Miyajima, J. Phys. Chem. B 105 (2001) 7920–7927.
[4] K. Inoue, T. Yamauchi, T. Itoh, E. Ihara, J. Inorg. Organomet. Polym Mater. 17 (2007) 367–375.
[5] J.F. Kuan, K.F. Lin, J. Appl. Polym. Sci. 91 (2004) 697–702.
[6] J. Ding, H. Liang, W. Shi, X. Shen, J. Appl. Polym. Sci. 97 (2005) 1776–1782.
[7] R. Liu, X. Wang, Polym. Degrad. Stab. 94 (2009) 617–624.
[8] G.X. Xu, Q. Lu, B.T. Yu, L. Wen, Solid State Ionics 177 (2006) 305–309.
[9] C.W. Allen, J. Fire Sci. 11 (1993) 320–328.
[10] K. Koran, F. Özen, G. Torğut, G. Pıhtılı, E. Çil, A.O. Görgülü, M. Arslan, Polyhedron 79 (2014) 213–220.
[11] L.S. Nair, S. Bhattacharyya, J.D. Bender, Y.E. Greish, P.W. Brown, H.R. Allcock, C.T. Laurencin, Biomacromolecules 5 (2004) 2212–2220.
[12] Y.E. Greish, J.D. Bender, S. Lakshmi, P.W. Brown, H.R. Allcock, C.T. Laurencin, Biomaterials 26 (2005) 1–9.
[13] K. Koran, A. Ozkaya, F. Ozen, E. Cil, M. Arslan, Res. Chem. Intermed. 39 (2013) 1109–1124.
[14] E.E. İlter, N. Asmafiliz, Z. Kılıç, L. Açık, M. Yavuz, E.B. Bali, A.O. Solak, F. Büyükkaya, H. Dal, T. Hökelek, Polyhedron 29 (2010) 2933–2944.
[15] A.İ. Öztürk, Ö. Yılmaz, S. Kırbağ, M. Arslan, Cell Biochem. Funct. 00 (2000) 117–126.
[16] N. Asmafiliz, Z. Kılıç, Z. Hayvalı, L. Açık, T. Hökelek, H. Dal, Y. Öner, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 86 (2012) 214–223.
[17] Ö. Yılmaz, F. Aslan, A.İ. Öztürk, N.S. Vanli, S. Kırbağ, M. Arslan, Bioorg. Chem. 30 (2002) 303–314.
[18] S.B. Koçak, S. Koçoğlu, A. Okumuş, Z. Kılıç, A. Özturk, T. Hökelek, Y. Öner, L. Açık, Inorg. Chim. Acta 406 (2013) 160–170.
[19] M. Siwy, D. Sęk, B. Kaczmarczyk, I. Jaroszewicz, A. Nasulewicz, M. Pelczyñska, D. Nevozhay, A. Opolski, J. Med. Chem. 49 (2006) 806–810.
[20] S. Tekin, K. Koran, F. Ozen, S. Sandal, A.O. Gorgulu, Acta Physiol. 211 (2014) 74.
[21] S.C. Song, S.B. Lee, B.H. Lee, H.W. Ha, K.T. Lee, Y.S. Sohn, J. Control. Release 90 (2003) 303–311.
[22] Y.J. Jun, J.I. Kim, M.J. Jun, Y.S. Sohn, J. Inorg. Biochem. 99 (2005) 1593–1601.
[23] S.S. Machakanur, B.R. Patil, G.N. Naik, R.P. Bakale, S.W.A. Bligh, K.B. Gudasi, Inorg. Chim. Acta 421 (2014) 459–464.
[24] Y. Tümer, N. Asmafiliz, Z. Kılıç, T. Hökelek, L.Y. Koç, L. Açık, M.L. Yola, A.O. Solak, Y. Öner, D. Dündar, M. Yavuz, J. Mol. Struct. 1049 (2013) 112–124.
[25] A.K. Andrianov, Polyphosphazenes for Biomedical Applications, Wiley, New Jersey, 2009.
[26] N. Asmafiliz, Z. Kılıç, T. Hökelek, L.Y. Koç, L. Açık, Y. Süzen, Y. Öner, Inorg. Chim. Acta 400 (2013) 250–261.
[27] H. Akbaş, A. Okumuş, Z. Kılıç, T. Hökelek, Y. Süzen, L.Y. Koç, L. Açık, Z.B. Çelik, Eur. J. Med. Chem. 70 (2013) 294–307.
[28] D. Hwang, J. Huyn, G. Jo, D. Koh, Y. Lim, Magn. Reson. Chem. 49 (2011) 41–45.
[29] A. Modzelewska, C. Pettit, G. Achanta, N.E. Davidson, P. Huang, S.R. Khan, Bioorg. Med. Chem. 14 (2006) 3491–3495.
[30] A.M. Asiri, S.A. Khan, Mater. Lett. 65 (2011) 1749–1752.
[31] C.G. Niu, A.L. Guan, G.M. Zeng, Y.G. Liu, Z.W. Li, Anal. Chim. Acta 577 (2006) 264–270.
[32] V.S. Pandey, R. Dhar, A.K. Singh, A.S. Achalkumar, C.V. Yelamaggad, Phase Transitions 83 (2010) 1049–1058.
[33] E.D. D'silva, D.N. Rao, R. Philip, R.J. Butcher, Rajnikant, S.M. Dharmaprakash, J. Phys. Chem. Solids 72 (2011) 824–830.
[34] A. Detsi, M. Majdalani, C.A. Kontogiorgis, D.H. Litina, P. Kefalas, Bioorg. Med. Chem. 17 (2009) 8073–8085.
[35] S. Bondock, T. Naser, Y.A. Ammar, Eur. J. Med. Chem. 62 (2013) 270–279.
[36] L. Mishra, R. Sinha, H. Itokawa, K.F. Bastow, Y. Tachibana, Y. Nakanishi, N. Kilgore, K.H. Lee, Bioorg. Med. Chem. 9 (2001) 1667–1671.
[37] M.V. Kaveri, R. Prabhakaran, R. Karvembu, K. Natarajan, Spectrochim. Acta Part A 61 (2005) 2915–2918.
[38] F. Herencia, M.L. Ferrándiz, A. Ubeda, J.N. Dominguez, J.E. Charris, G.M. Lobob, M.J. Alcaraz, Bioorg. Med. Chem. Lett. 8 (1998) 1169–1174.
[39] F. Hayat, E. Moseley, A. Salahuddin, R.L.V. Zyl, A. Azam, Eur. J. Med. Chem. 46 (2011) 1897–1905.
[40] S.H. Kim, E. Lee, K.H. Baek, H.B. Kwon, H. Woo, E.S. Lee, Y. Kwon, Y. Na, Bioorg. Med. Chem. Lett. 23 (2013) 3320–3324.
[41] C. Jin, Y.J. Liang, H. He, L. Fu, Biomed. Pharmacother. 67 (2013) 215–217.
[42] O. Sabzevari, G. Galati, M.Y. Moridani, A. Siraki, P.J. O'Brien, Chem. Biol. Interact. 148 (2004) 57–67.
[43] A. Kamal, G. Ramakrishna, P. Raju, A. Viswanath, M.J. Ramaiah, G. Balakishan, M.P. Bhadra, Bioorg. Med. Chem. Lett. 20 (2010) 4865–4869.
[44] H.I. Gul, K.O. Yerdelen, M. Gul, U. Das, B. Pandit, P.K. Li, H. Secen, F. Sahin, Arch. Pharm. Chem. Life Sci. 340 (2007) 195–201.
[45] E. Çil, M. Arslan, A.O. Görgülü, Polyhedron 25 (2006) 3526–3532.
[46] E. Çil, M. Arslan, Inorg. Chim. Acta 362 (2009) 1421–1427.
[47] E. Çil, M. Arslan, A.O. Görgülü, Heteroat. Chem. 17 (2006) 112–117.
[48] E. Çil, M. Arslan, A.O. Görgülü, Can. J. Chem. 83 (2005) 2039–2045.
[49] H.A. Alidağı, B. Çoşut, A. Kılıç, S. Yeşilot, Polyhedron 81 (2014) 436–441.
[50] E.W. Ainscough, A.M. Brodie, G.B. Jameson, C.A. Otter, Polyhedron 26 (2007) 460–471.
[51] S. Ladislav, Z. Jozefína, P. Nadezda, Molecules 2 (1997) 7–10.
[52] M. Sathishkumar, P. Shanmugavelan, S. Nagarajan, M. Maheswari, M. Dinesh, A. Ponnuswamy, Tetrahedron Lett. 52 (2011) 2830–2833.
[53] L. Kapička, P. Kubáček, P. Holub, J. Mol. Struct. (Thoechem) 820 (2007) 148–158.
[54] Z. Ngaini, N.I. Abdul Rahman, Can. J. Chem. 88 (2010) 654–658.
[55] Z. Ngaini, N.I. Abdul Rahman, Phosphorus, Sulfur Silicon Relat. Elem. 185 (2010) 628–633.
[56] H.R. Allcock, C.G. Cameron, Macromolecules 27 (1994) 3131–3135.
[57] G.A. Carriedo, L.F. Catuxo, F.J.G. Alonso, P.G. Elipe, P.A. González, Macromolecules 29 (1996) 5320–5325.
[58] S. Tekin, S. Sandal, C. Colak, Med. Sci. 3 (2014) 1427–1441.
[59] B. Yilmaz, S. Sandal, C.H. Chen, D.O. Carperter, Toxicology 217 (2006) 184–193.
[60] T.R. Mosamann, H. Cherwinski, M.V. Bond, M.A. Giedliv, R.F. Coffmann, J. Immunol. 136 (1986) 2348–2355.
[61] N.K. Singh, S.B. Singh, Synth. React. Inorg. Met. – Org. Chem. 32 (2002) 25–47.